PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 041117woMenn	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/EP2004/009043	International filing date *(day/month/year)* 12.08.2004	Priority date *(day/month/year)* 12.08.2003
International Patent Classification (IPC) or national classification and IPC C07K14/81, A61L2/00		
Applicant OCTAPHARMA AG et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10.06.2005	12.09.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Stolz, B Telephone No. +49 89 2399-8416

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/009043

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-14	as originally filed
Claims, Numbers	
1-19	filed with the demand
Drawings, Sheets	
1/1	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/009043

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11
	No:	Claims	12-19
Inventive step (IS)	Yes:	Claims	1-11
	No:	Claims	12-19
Industrial applicability (IA)	Yes:	Claims	1-19
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

1. The application discloses a process for purifying A1AT from A1AT-containing solutions from other protein components comprising the steps of a) ion exchange chromatography, b) virus inactivation by detergent and optionally additional virus inactivating substances, and c) salting out of the detergent. The effect of this procedure is not only removal of the detergent and solvent but also removal of contaminating proteins.

2. Novelty (Art. 33(2) PCT)

   Methods for purifying A1AT from contaminating proteins comprising the sequence of steps of claim 1 have not been disclosed in the prior art. Hence claim 1 and dependent claims 2 to 11 are novel.

   Product claims 12 to 19 lack novelty in view of the A1AT preparations disclosed in EP436086, DE4407837 and WO98/56821. All three documents disclose A1AT of high purity. Example 2 of WO98/56821 uses an A1AT produced by SERVA which according to the manufacturers specification is already 95% pure and has an activity of 0.81 PEU. According to Table 2, the product obtained by the method of Expl. 2 is >100% pure and has a specific activity of 1 PEU. Experimental evidence about the IgA content of the preparations obtained according to the three cited documents is not available. Therefore, also the products of EP436086 and DE4407837 are considered to be falling within the terms of the claims. Claims 13 to 17 lack novelty because they are directed to products obtainable by the claimed method, i.e. they are directed to the products per se.

3. Inventive step (Art. 33(3) PCT)

   The technical problem solved by the present application is the provision of an alternative system for the purification of A1AT containing solutions from contaminating proteins.

   This problem is solved by the method of claim 1.

   Many procedures for purifying A1AT containing solutions have been known in the art.

Most of them use at least one step of ion exchange and a step for virus inactivation by the addition of a detergent/solvent system (e.g. WO98/56821 or DE 4407837). Additional purification steps known in the art involve hydrophobic interaction chromatography, affinity chromatography with heparin (US4629567) or Affi-Gel columns (EP436086). Thus, a number of alternative purification procedures were conceivable. None of them involved a step of salting out detergents. Using any of the above cited documents, the person of skill could therefore not have arrived at the claimed solution with a reasonable expectation of success.

The salting out of detergents for removing inactivated viruses from preparations of plasma proteins has been disclosed in WO94/26287. However, the purpose of the disclosed method is the removal of detergent and solvent and not the removal of contaminating proteins. This document does not disclose any effect of the salting out on the removal of contaminating proteins. Thus, when trying to solve the above mentioned problem, the person of skill would not have expected the method disclosed in WO94/26287 to lead to an improvement in protein purification. He would not have combined the teaching of WO94/26287 with any of the above mentioned documents with a reasonable expectation of success.

Hence, claims 1 to 11 are considered to involve an inventive step.

CLAIMS:

1. A process for ~~preparing~~ purifying * <—> A1AT from A1AT-containing solutions, comprising the following steps:

   (a) subjecting an A1AT-containing solution to ion-exchange chromatography;

   ~~(b) adding detergents and optionally a solvent for inactivating lipid~~ enveloped viruses;

   (c) followed by increasing the salt concentration to salt out the detergents.

2. The process according to claim 1, wherein said A1AT-containing solution has been obtained from blood plasma or its fractions, preferably from a reconstituted plasma fraction IV1 (Cohn), or is derived from a recombinantly or transgenically expressed A1AT preparation or a fermentation supernatant.

3. The process according to claim 1 and/or 2, wherein ion-exchange chromatography is performed on an anion-exchange gel, preferably DEAE-Sepharose® or DEAE-Sepharose® Fast Flow.

4. The process according to any of claims 1 to 3, wherein said virus inactivation according to step (b) is effected with Triton X-100, Polysorbate 80 (Tween 80), TnBP and/or caprylic acid or caprylate, preferably at final concentrations of ≥ 0.1% (w/w) Triton and Tween 80, ≥ 0.03% (w/w) TnBP, ≥ 0.1 mM caprylic acid or caprylate, with an incubation time of ≥ 0.1 hours, preferably ≥ 1 hour, at ≥ 4 °C, especially at ≥ 15 °C.

* < from other protein components >

5. The process according to any of claims 1 to 4, wherein the salt concentration of the solution is brought to ≥ 0.5 M in step (c) and particles formed thereby are preferably removed by filtration.

6. The process according to any of claims 1 to 5, wherein chromatography on hydrophobic chromatographic materials is performed.

7. The process according to any of claims 1 to 6, wherein a treatment of the A1AT-containing fraction with a material which contains heparin in an immobilized form (heparin gel) is performed.

8. The process according to any of claims 5 to 7, wherein a further virus inactivation step is performed afterwards, preferably pasteurization in the presence of ≥ 0.5 M sodium citrate, amino acids, sugars or mixtures thereof.

9. The process according to any of claims 1 to 8, wherein the ion strength of the solution is preferably reduced by ultra-/diafiltration.

10. The process according to any of claims 1 to 9, wherein a separation of virus particles is performed, preferrably by nanofiltration, preferably with filters having pore sizes of 15-20 nm.

11. The process according to any of claims 1 to 10, wherein the A1AT fraction obtained is stored as a liquid, frozen or freeze-dried preparation.

12. A1AT having a purity of > 90%, an activity of ≥ 0.8 PEU/mg in its active form, an IgA content of ≤ 1 mg/ml, a residual detergent content of < 50 ppm, especially < 10 ppm, and a monomer content of > 90%, based on the total amount of A1AT.

13. The A1AT according to claim 12, obtainable by a process comprising the following steps:

- reconstitution of plasma fraction IV1 (Cohn);
- anion-exchange chromatography on DEAE-Sepharose® Fast Flow;
- optionally chromatography on a solid phase which comprises heparin in an immobilized form (heparin affinity chromatography);
- optionally hydrophobic interaction chromatography (HIC);

- virus inactivation with ≥ 0.1% (w/w) Triton/≥ 0.03% (w/w) TnBP with an incubation time of ≥ 1 hour at ≥ 15 °C;
- addition of salt to increase the ion strength of the solution; and
- removal by filtration of particles formed thereby.

14. The A1AT according to claim 13, wherein a further virus inactivation step is performed afterwards, preferably pasteurization in the presence of ≥ 0.5 M sodium citrate, amino acids, sugars or mixtures thereof.

15. The A1AT according to claim 13, wherein the ion strength of the solution is preferably reduced by ultra-/diafiltration.

16. The A1AT according to claim 13, wherein a virus and/or prion depletion or inactivation step is comprised, preferably a separation of virus particles by nanofiltration, preferably with filters having pore sizes of 15-20 nm.

17. The A1AT according to claim 13, wherein the A1AT fraction obtained is stored as a liquid, frozen or freeze-dried preparation.

18. A medicament containing an A1AT according to any of claims 12 to 17 as a sole active ingredient or in combination with anti-inflammatory agents, preferably steroids, NSAIDs.

19. Use of the A1AT according to any of claims 12 to 17 for preparing a medicament for the treatment of A1AT deficiency, degenerative phenomena of the lung, such as lung fibrosis and emphysema.